

Stepwise metamorphosis of the tubeworm *Hydroides elegans* is mediated by a bacterial inducer and MAPK signaling

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Diverse animal taxa metamorphose between larval and juvenile phases in response to bacteria. Although bacteria-induced metamorphosis is widespread among metazoans, little is known about the molecular changes that occur in the animal upon stimulation by bacteria. Larvae of the tubeworm *Hydroides elegans* metamorphose in response to surface-bound *Pseudoalteromonas luteoviolacea* bacteria, producing ordered arrays of phage tail-like metamorphosis-associated contractile structures (MACs). Sequencing the *Hydroides* genome and transcripts during five developmental stages revealed that MACs induce the regulation of groups of genes important for tissue remodeling, innate immunity, and mitogen-activated protein kinase (MAPK) signaling. Using two MAC mutations that block *P. luteoviolacea* from inducing settlement or metamorphosis and three MAPK inhibitors, we established a sequence of bacteria-induced metamorphic events: MACs induce larval settlement; then, particular properties of MACs encoded by a specific locus in *P. luteoviolacea* initiate cilia loss and activate metamorphosis-associated transcription; finally, signaling through p38 and c-Jun N-terminal kinase (JNK) MAPK pathways alters gene expression and leads to morphological changes upon initiation of metamorphosis. Our results reveal that the intricate interaction between *Hydroides* and *P. luteoviolacea* can be dissected using genomic, genetic, and pharmacological tools. *Hydroides*' dependency on bacteria for metamorphosis highlights the importance of external stimuli to orchestrate animal development. The conservation of *Hydroides* genome content with distantly related deuterostomes (urchins, sea squirts, and humans) suggests that mechanisms of bacteria-induced metamorphosis in *Hydroides* may have conserved features in diverse animals. As a major biofouling agent, insight into the triggers of *Hydroides* metamorphosis might lead to practical strategies for fouling control.

genome | phage | symbiosis | biofouling | development

Free-swimming larvae of marine invertebrates must identify sites favorable for their settlement and subsequent metamorphosis into adults. But how do they identify these sites? Many species—including sponges (1), corals (2, 3), crabs (4), sea urchins (5), and ascidians (6)—use cues from bacteria (Fig. 1A) (7). This bacteria-mediated metamorphosis is essential for coral reef formation (8), and causes the costly accumulation of encrusting organisms on submerged surfaces such as the hulls of ships [i.e., biofouling (7, 8)]. Although in a few cases the bacterial cues (and the genes encoding them) have been identified (3, 9, 10), very little is known about how these cues mediate the resultant developmental cascade in animals and how developmental cues may be manipulated to promote or deter colonization.

Metamorphosis can be defined as the morphological, physiological, and behavioral transition from larva to juvenile (11, 12). During bacteria-induced metamorphosis, the sensing of bacterial cues must be coordinated with the regulation of development (7, 11, 12). It is hypothesized that metamorphosis evolved multiple times among metazoans, yet common signaling systems were independently coopted to coordinate the metamorphic transition

(12–15). These systems include hormones (16–18), neurotransmitters (19–21), and nitric oxide (22–24). Additionally, diverse animals regulate metamorphosis at the transcriptional and posttranslational levels, such as differential expression of metamorphosis-associated genes (25–27) and MAPK signaling (28–31), respectively. Although a number of signaling systems and regulatory networks orchestrate metamorphosis in animals from diverse taxa, comparatively little is known about how bacteria stimulate these systems and networks.

The tubeworm *Hydroides elegans* (Haswell 1883; hereafter *Hydroides*) is a significant biofouling pest in tropical and subtropical harbors (32, 33), and its larvae are dependent on bacteria to initiate metamorphosis (9, 10, 34, 35). This makes it an appealing model organism in which to study the mechanisms of biofouling and how bacteria mediate animal development. In the laboratory, only certain bacterial species induce tubeworm metamorphosis (34–36), suggesting that some bacteria possess particular properties that serve as a metamorphosis cue. One such bacterium is *Pseudoalteromonas*

Significance

Free-swimming larvae of many animals that inhabit the sea floor metamorphose in response to bacteria. However, the molecular mechanisms that underpin animal metamorphosis in response to bacterial triggers remain elusive. We investigated the developmental cascade induced by bacteria in a model tubeworm, *Hydroides elegans*, and identified a bacterial mutant and host signaling system critical for the initiation of and tissue remodeling during metamorphic development, respectively. Identifying the triggers for metamorphosis in *Hydroides* has implications for understanding basic principles of bacteria–animal interactions. Such interactions are also major causes of biofouling, which our research indicates may be controlled by manipulating bacterial inducers or MAPK activities that are essential for tubeworm metamorphosis.

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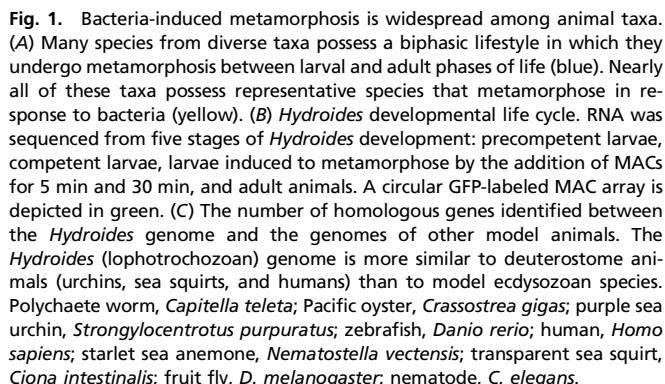
Data deposition: The raw sequence reads reported in this paper have been deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) database [accession nos. SRP067980 (genome) and SRP067899 (transcriptome)]. The whole-genome shotgun project reported in this paper has been deposited in the DNA Data Bank of Japan (DDBJ)/European Molecular Biology Laboratory (EMBL)/GenBank database (accession no. LQRL00000000, version LQRL01000000).

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To investigate the *Hydroides* developmental cascade induced by MACs, we used two bacterial mutations that block *Hydroides* settlement or metamorphosis at different stages. Sequencing *Hydroides* transcripts during five developmental stages identified groups of gene products that were key to metamorphosis. One such group comprised MAPK signaling proteins, whose roles were verified using inhibitors of MAPK phosphotransfer activity, indicating that metamorphosis relies on posttranslational modifications. These genomic, genetic, and pharmacological tools enabled us to dissect the sequence of events following the induction of tubeworm metamorphosis by a bacterial inducer.

The *Hydroides* Genome Possesses a Conserved Gene Content. To investigate the events that occur upon bacteria-stimulated metamorphosis, we sequenced the genome of *Hydroides* (48× average coverage) and transcripts at five developmental stages: (i) pre-competent larvae; (ii) competent larvae (larvae capable of completing metamorphosis); competent larvae exposed to purified MACs for (iii) 5 min and (iv) 30 min; and (v) adult animals (Fig. 1B). The assembled draft genome is 1,026.1 Mb with an N50 (weighted median contig size) of 17.3 kb, and is predicted to encode 142,653 transcripts, corresponding to 113,410 loci, of which 93,636 are protein-coding; 33.94% of the genome is represented by repetitive sequences (*SI Appendix, Table S1*). Using the Core Eukaryotic Genes Mapping Approach (CEGMA) (42), the completeness of the assembly is estimated to be 89.1%. Additional information on the *Hydroides* genome and transcriptome can be found in *SI Appendix, Results*.

To gauge the relative gene conservation of *Hydroides* in relation to other model species that represent the breadth of multicellular animal life, we mapped all *Hydroides* genes against the genomes of several model organisms. *Hydroides* (a lophotrochozoan) and the fruit fly *Drosophila melanogaster* and nematode *Caenorhabditis elegans* (both ecdysozoans) group into the protostome clade (Fig. 1A). However, the *Hydroides* genome possesses more gene homologs with deuterostomes (urchins, sea squirts, zebrafish, and humans) and a nonbilaterian cnidarian (sea anemone) than it does with these model ecdysozoans (Fig. 1C). We further classified *Hydroides* genes into major metazoan groupings based on their homology [BLASTP against the non-redundant (nr) database, *e*-value cutoff of 10^{-10} ; *SI Appendix, Fig. S1*]. Of *Hydroides'* 61,540 genes with identified homologs, 6,087 genes (9.9%) are shared with deuterostomes and nonbilaterian animals only and not ecdysozoans. In comparison, 112 (0.2%) are shared with ecdysozoans and nonbilaterians only and not deuterostomes. These results suggest that *Hydroides* genome content has not evolved as quickly as the fruit fly and nematode genomes. Consistent with these findings, other annelids were found to possess relatively slowly evolving genomes (43, 44), and *D. melanogaster* and *C. elegans* are documented to possess more derived genomes with extensive gene loss and higher rates of molecular evolution (45, 46). Studying mechanisms that mediate bacteria-induced metamorphosis in *Hydroides* might provide a framework to understand bacteria–animal interactions in diverse animals.

Two Bacterial Loci Are Required for Different Stages of Metamorphosis.

Settlement and metamorphosis of *Hydroides* in response to MACs can be classified into five stages: (i) swimming; (ii) initiation of settlement, indicated by slow swimming or crawling upon contact with MACs; (iii) irreversible initiation of metamorphosis, starting with the loss of cilia near the prototroch; (iv) body elongation and primary tube secretion; and (v) eversion of the collar, production of the secondary tube, and formation of branchial lobes (Movie S1). We quantified settlement by observing the vertical distribution of larvae at 5 and 30 min after exposure to MACs. Five minutes after larvae were exposed to wild-type MACs, larvae initiated settlement behavior (i.e., slowed or stopped swimming), causing them to collect at the bottom of the chamber (Fig. 2A). Larvae exposed to extract from a bacterial strain lacking the baseplate of MACs ($\Delta macB$), a key structural component of MACs (9, 10), did not initiate settlement behavior or begin metamorphosis.

When exposed to MACs from a *P. luteoviolacea* mutant lacking a segment of the genome containing six genes (JF50_12590–JF50_12615, ~8.2 kb; hereafter $\Delta 12590$ –615), *Hydroides* larvae initiated settlement behavior but did not lose their cilia or continue to metamorphose compared with larvae exposed to wild-type MACs (Movie S2). After 5 min, these larvae were distributed significantly differently from larvae unexposed to MACs or larvae exposed to extract from a $\Delta macB$ strain (Fig. 2A). Thirty minutes after exposure, these larvae resumed a distribution similar to unexposed or $\Delta macB$ controls, whereas larvae exposed to wild-type MACs continued to metamorphose (Fig. 2B). All six genes deleted in the $\Delta 12590$ –615 mutant encode hypothetical proteins and lie about 12 kb away from the closest characterized gene (*macS*) known to be essential for the structure and function of MACs (10). When these six genes were reintroduced into their original genomic location, the bacteria were again able to induce metamorphosis (Fig. 2C). MACs from the $\Delta 12590$ –615 mutant were similar to MACs from *P. luteoviolacea* wild type in number (Fig. 2D), size, and structure (Fig. 2E–G). These results suggest that the structure of MAC arrays produced by the $\Delta 12590$ –615 mutant was largely intact and sufficient to initiate settlement. However, the deleted region is required for MACs to initiate the metamorphosis of *Hydroides*.

MACs Activate Tissue Remodeling-, Innate Immunity-, and Signaling-Associated Genes upon Stimulation of Metamorphosis. To determine whether changes in gene expression accompany the initial events of *Hydroides* settlement and metamorphosis, we compared the transcriptomes of competent larvae unexposed to MACs with larvae exposed to MACs for 5 or 30 min. [These time points were chosen because at these times, larvae clearly responded differently to MACs from the wild-type and $\Delta 12590$ –615 strains (Fig. 2).] When we compared transcripts from competent larvae unexposed to MACs with larvae exposed to MACs for 5 min, no transcripts were identified as significantly different. However, by 30 min, exposure to MACs resulted in significantly different transcription of 156 genes ($P \leq 0.05$; 8 down-regulated and 148 up-regulated; Dataset S1).

Numerous genes were up-regulated upon the stimulation of metamorphosis with bacterial phage tail-like structures (SI Appendix, Fig. S2 and Dataset S1). Notably, MACs stimulated the expression of five astacin-family (47) matrix metalloproteinase (MMP)-encoding genes 4.9- to 21.0-fold after 30 min of induction and a collagen triple-helix (PF01391) domain-containing protein (up-regulated 6.0-fold). The up-regulation of MMP and collagen domain-containing genes is consistent with enzyme-mediated remodeling of tissues and structures accompanying metamorphosis (48). MACs stimulated the expression of two kazal-type serine protease inhibitor (SPI) domain-containing proteins (up-regulated 3.0- and 3.1-fold), known to have antimicrobial properties and implicated in host innate immunity in other invertebrate animals (49, 50). MACs induced the expression of 15 genes (up to 552.3-fold) encoding proteins with von Willebrand factor (VWF) domains, which can play important roles in cell adhesion (51).

Nine genes encoding ankyrin repeats were up-regulated (up to 110.2-fold), which are often involved in protein–protein interactions (52). MACs stimulated a significant number of genes encoding nucleic acid-binding transcription factors, including a set that is typically downstream of MAPK signaling pathways (up-regulated 2.2- to 10.0-fold). Two genes encoding proteins with similarity to tumor necrosis factor receptor-associated factor 3, shown to function in MAPK signaling complexes in humans (53), were up-regulated 42.5- and 56.6-fold in response to MACs after 30 min. These results demonstrate that after 30 min of exposure to MACs, transcription of distinct sets of tissue remodeling-, innate immunity-, and signal transduction-associated genes was dramatically increased.

p38 and JNK MAPK Signaling Regulate Steps of Metamorphosis After Cilia Loss.

To understand the events following MAC-induced development, we asked whether MAPK signaling is important for *Hydroides* metamorphosis. We confirmed the presence of three extensively studied subgroups of MAPK genes (ERK, JNK, and p38) and associated signaling components in the *Hydroides* genome (Fig. 3A and SI Appendix, Table S2). Transcript abundances of most of the MAPK genes from competent larvae were high enough (>1 fragments per kb of transcript per million mapped reads) (SI Appendix, Table S2) to indicate that these genes were expressed upon induction of metamorphosis. We then asked whether metamorphosis proceeds in the presence of chemical inhibitors against each MAPK pathway. The U0126 inhibitor against mitogen/extracellular signal-regulated kinase (MEK; a kinase of ERK) did not prevent metamorphosis in response to MACs (Fig. 3B). However, the production of a primary and a secondary tube, larval body elongation, and development of branchial radioles were inhibited when larvae were pretreated for 2 h with the p38 inhibitor SB203580 or the JNK inhibitor SP600125 (Fig. 3C–E). Although these inhibitors abrogated some developmental changes, larvae initiated settlement behavior (stopped swimming) and metamorphosis (lost cilia localized to the prototroch). Without the addition of MACs, the larvae appeared normal after 24 h in the presence of up to 10 μ M all inhibitors tested, suggesting that the concentration of inhibitors used was not toxic. To determine whether the inhibitors were active against their intended targets, we measured the levels of specific phosphorylated components of the p38 and JNK pathways using antibodies raised against mammalian phosphoproteins with homologs in *Hydroides* (SI Appendix, Table S2). *Hydroides* protein extracts had increased levels of diphosphorylated p38 after treatment with SB203580 and decreased levels of diphosphorylated JNK after SP600125 treatment (SI Appendix, Fig. S3), similar to previous findings in human cells (54, 55). Upon stimulation, p38 phosphorylates MAPKAPK-2 and ATF-2, both of which were moderately increased after stimulation by MACs but not in the presence of SB203580 (SI Appendix, Fig. S3). Similarly, JNK phosphorylation of ATF-2 and p53 increased after exposure to MACs but was inhibited by SP600125 (SI Appendix, Fig. S3). These results identify p38 and JNK MAPK pathways as important phosphotransfer signaling systems in *Hydroides* metamorphic development.

A Bacterial Locus Responsible for Metamorphosis-Associated Transcription.

We then sought to identify the developmental stage at which metamorphosis-associated transcription could be detected. Quantitative real-time PCR (qRT-PCR) robustly detected the up-regulation of Fos (TCONS_00100867) and VWF (TCONS_00123609) genes in response to wild-type MACs after 30 min (Fig. 3F and SI Appendix, Fig. S3). Pretreatment of larvae with the p38 and JNK inhibitors SB203580 or SP600125 partially repressed Fos and VWF expression compared with controls induced to metamorphose by MACs without an inhibitor (SI Appendix, Fig. S3). These results suggest that p38 and JNK signaling might act downstream of or in parallel to an initial signal of metamorphosis.

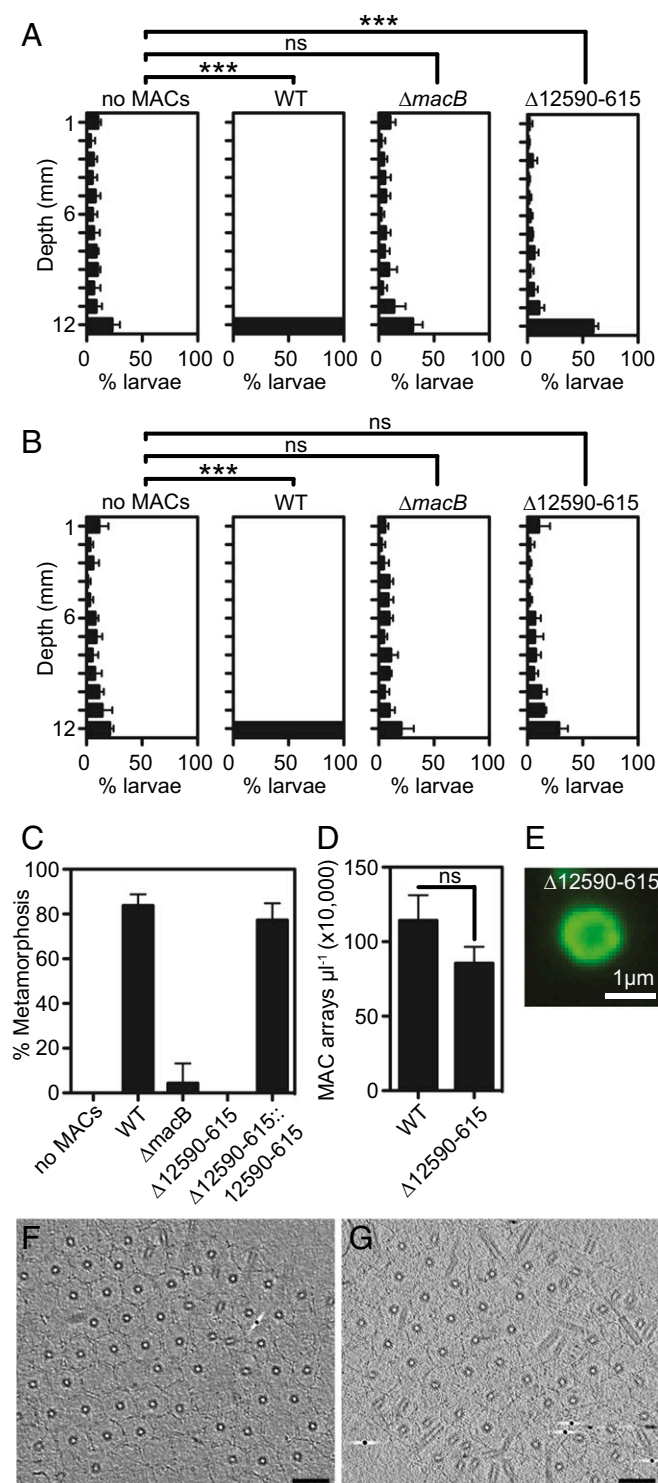


Fig. 2. Two bacterial loci are required for different stages of metamorphosis. (A and B) MAC extract from wild-type, $\Delta macB$, or $\Delta 12590-615$ strains was added to competent larvae in polystyrene cuvettes in a total of 0.5 mL. The vertical distribution of larvae within the cuvette was quantified at 5 min (A) or 30 min (B) after addition of MACs. Artificial seawater without MACs served as a negative control. Error bars indicate SDs of six biological replicates. Twelve to 55 larvae were quantified per replicate. Student's *t* test of mean depth: ns, not significant; ****P* < 0.00001. (C) Metamorphosis of *Hydroides* exposed to MAC extract from wild-type, $\Delta 12590-615$, $\Delta macB$, or $\Delta 12590-615::12590-615$. Error bars are SDs of four biological replicates. (D) Quantification of MAC arrays from extract of wild type or $\Delta 12590-615$. Error bars are SDs of three biological replicates. Student's *t* test. (E) Image of a fluorescently tagged MAC from

We then tested whether transcriptional changes occur coincident with the initiation of settlement or metamorphosis by using $\Delta 12590-615$ MACs or wild-type MACs, respectively, as inducers. Expression of Fos and VWF, measured by qRT-PCR, was not significantly activated in response to $\Delta 12590-615$ MACs compared with wild type (Fig. 3F), suggesting that metamorphosis-associated transcription and the activation of MAPK signaling require the JF50_12590-JF50_12615 genomic region of *P. luteoviolacea*. These results show that metamorphosis-associated transcription and MAPK signaling are activated coincident with the loss of cilia and establish a timeline of inductive and signaling events following MAC-initiated metamorphosis of *Hydroides*: MACs engage with tubeworm larvae, inducing them to initiate settlement behavior; then, particular properties or modification of MACs encoded by the JF50_12590-JF50_12615 region initiate metamorphosis (cilia loss) and activate metamorphosis-associated transcription; and, finally, p38 and JNK signaling are partially responsible for the regulation of gene expression and morphological remodeling events after cilia loss (Fig. 3G).

Discussion

We now know that interactions with bacteria can directly benefit animals (56, 57), and one of the most dramatic examples occurs when bacteria induce animal metamorphosis. Although representative species from diverse taxa undergo bacteria-induced metamorphosis (Fig. 1A), little is known about the processes that occur within the animal as they detect the cue produced by bacteria. To understand the developmental cascade in animals after bacteria induce metamorphosis, we sequenced the genome of *Hydroides* and analyzed changes in gene expression upon exposure to MACs. Although *Hydroides* (a lophotrochozoan), fruit flies, and nematodes (ecdysozoans) phylogenetically group as protostomes (58), the *Hydroides* genome shares more gene homologs with deuterostome (purple sea urchin, zebrafish, and human) and nonbilaterian (starlet sea anemone) genomes (Fig. 1C and SI Appendix, Fig. S1). Previous studies also reported relatively slow evolutionary rates of annelids compared with the model ecdysozoans *D. melanogaster* and *C. elegans* (43–46), suggesting that annelids might share more ancestral characteristics with distantly related model animals. Mechanisms underpinning the metamorphic response of *Hydroides* to bacterial inducers might therefore have conserved features present in other metazoans and serve as an example of how bacteria impact the development of an animal. In an applied context, such features are of interest because they might be used as targets for the general control of biofouling organisms.

In addition to the conservation of genome content, many genes that were differentially regulated upon the stimulation of *Hydroides* metamorphosis are homologous to genes associated with development in other model animals. *Hydroides* larvae up-regulated MMPs in response to MACs, important factors involved in the remodeling of tissue. The first MMP to be described was found to degrade fibrillar collagen in tadpole tails during metamorphosis (48). In the greater wax moth, both metamorphosis and collagen VI fragments were shown to stimulate an innate immune response (59), and this response is thought to be mediated by an endogenous MMP (60). *Hydroides* up-regulated SPI-encoding genes, which have potent bactericidal activity implicated in host innate immunity in *Hydra* (49) and black tiger shrimp (50). Similar differential expression of immunity genes during metamorphosis was observed in corals (25, 28) and ascidians (14, 27, 61). VWF domain-encoding genes were up-regulated and might be important for tube building. Proteins containing VWF domains were found in the organic

$\Delta 12590-615$ *macB-sfgfp*. (F and G) The wild type (F) and $\Delta 12590-615$ mutant (G) produce ordered arrays having comparable size and number of MACs. Shown are 9-nm slices through electron cryotomograms (Scale bars, 100 nm).

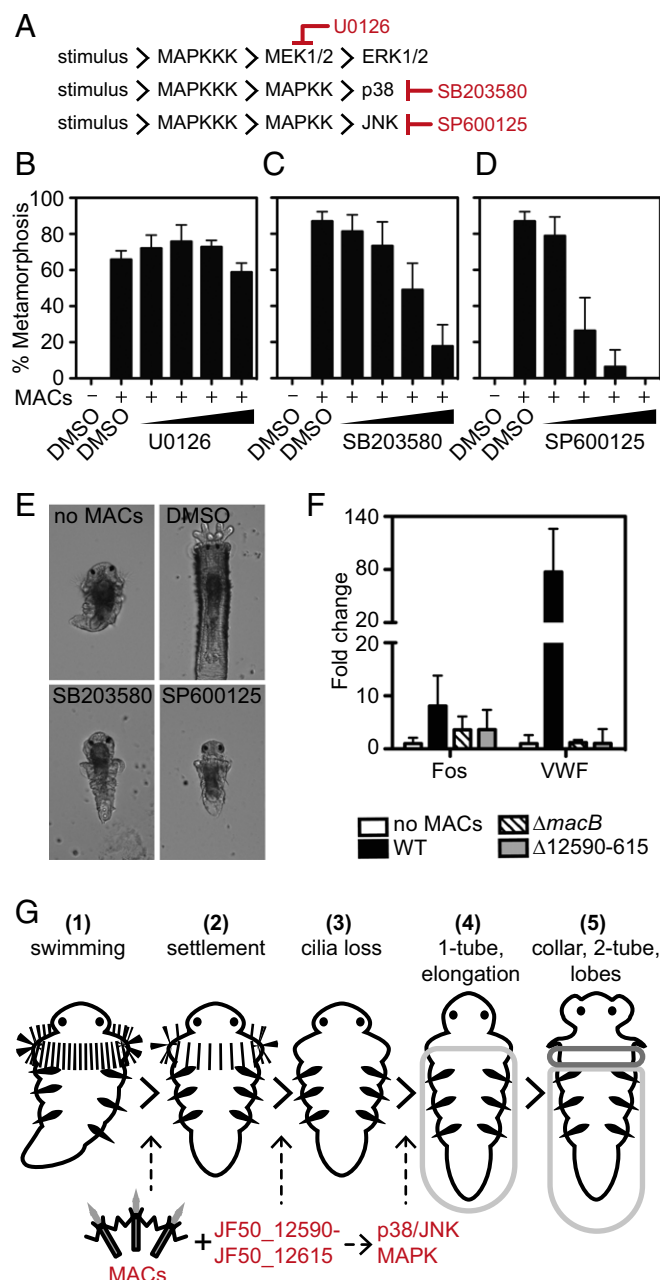


Fig. 3. Bacterial MACs and MAPK signaling regulate distinct steps of metamorphosis. (A) Components of the MAPK pathway identified in the genome of *Hydroides*. (B–D) Metamorphosis of *Hydroides* in the presence of the MEK inhibitor U0126 (B), p38 inhibitor SB203580 (C), or JNK inhibitor SP600125 (D). Larvae were pretreated with inhibitor for 2 h at 0.25, 1.0, 2.5, or 10 μ M and induced to metamorphose with MACs. Solvent-only (DMSO) treatments are shown as controls. Error bars indicate SDs of eight biological replicates. (E) Representative images of larvae pretreated with the p38 inhibitor SB203580 or JNK inhibitor SP600125 for 2 h and induced to metamorphose with MACs for 24 h. Larvae without inhibitor and MACs (no MACs) or larvae treated with solvent and MACs (DMSO) are shown. (F) Fold change of Fos (TCONS_00100867) and VWF (TCONS_00123609) genes from larvae after a 30-min exposure to MACs from wild-type, $\Delta macB$, or $\Delta 12590-615$ strains relative to unexposed larvae. (G) Timeline of metamorphic events in response to MACs: free-swimming competent larvae (1); MACs induce settlement behavior (2); with the JF50_12590–JF50_12615 region, MACs induce the irreversible initiation of metamorphosis (cilia loss) and activate metamorphosis-associated transcription (3); larvae produce a primary proteinaceous tube (1-tube) and their body elongates (4); larvae evert their collar, larvae produce a calcareous secondary tube (2-tube), and branchial lobes form (5). p38 and JNK MAPK signaling mediate body elongation, primary and secondary tube formation, and branchial lobe formation.

matrix of oyster shells (62) and coral skeletons (63). Alternatively, VWF domain-containing genes could be involved in cell adhesion during tissue remodeling (51). *Hydroides* larvae up-regulated MAPK signaling genes, which were previously implicated in modulating metamorphosis of other marine organisms including corals (28), ascidians (30), and barnacles (31). The transcriptional signature of metamorphosis in *Hydroides* therefore parallels developmental processes identified in other animal species.

To understand how bacteria activate animal development, we dissected the events occurring upon bacteria-stimulated metamorphosis. Although transcription changed following MAC exposure, the initial signal reception and transduction orchestrating *Hydroides* metamorphosis are likely initially mediated by post-translational mechanisms (64) and subsequently lead to the differential expression of metamorphosis-associated genes. Based on transcript sequencing and use of pharmacological inhibitors, we found that such posttranslational mechanisms include p38 and JNK MAPK signaling. A previous report showed the importance of p38 signaling in *Hydroides* metamorphosis in response to natural marine biofilms, but results were inconclusive for the JNK inhibitor SP600125 (29). Our results suggest that p38 and JNK signaling are necessary for the stages of MAC-induced metamorphosis involving morphological remodeling following cilia loss, including body elongation, primary and secondary tube formation, and branchial lobe formation (Fig. 3).

In addition to using genomics and pharmacological inhibitors, we used two MAC mutants to dissect metamorphosis; one is unable to initiate settlement ($\Delta macB$), and the other initiates settlement but does not induce metamorphosis ($\Delta 12590-615$). Using these mutants, we determined that the irreversible step of metamorphosis starts with cilia loss, activation of metamorphosis-associated gene expression, and MAPK signaling. We hypothesize that the JF50_12590–JF50_12615 region encodes structures for the proper binding of MACs to larvae and components delivered to larvae by the MAC structure, or mediates metamorphosis by encoding chaperones essential for the proper assembly or conformation of MACs. Future work will reveal the mechanisms by which these and other loci affect settlement and metamorphosis of *Hydroides*.

In conclusion, we sequenced the genome and transcriptome of a model tubeworm and show how its settlement and metamorphic program respond to a bacterial inducer. As a member of the undersampled lophotrochozoan clade, the availability of the *Hydroides* genome will broaden the repertoire of tools available to study metazoan evolution. Identifying the molecular basis of bacteria-mediated metamorphosis will help us understand the molecular coevolution of bacteria and animals, which may have played an important role in generating the spectacular diversity in animal form and function that we see today. Our results indicate that bacterial factors and intracellular signaling systems may be controlled during surface colonization for applications, such as the management of biofouling, and to better understand processes that govern the recruitment and maintenance of marine animal populations.

Methods

Specimens of *H. elegans* were obtained from Marina Del Rey, California. Embryos were obtained and maintained as previously described (10, 32). Two libraries with average insert sizes of 300 and 600 bp were prepared using the New England Biolabs, Inc. Next Ultra DNA Library Prep Kit (NEB; E7370) according to the manufacturer's instructions with minor modifications. Libraries were sequenced on an Illumina HiSeq 2500 instrument in paired-end mode with a read length of 150 nt to the depth of 100 million and 75 million reads for the 300- and 600-bp libraries, respectively. Detailed genome sequencing, assembly, and experimental procedures and datasets are provided in *SI Appendix, Methods, and Datasets S2–S4*.

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